

## **CLAIM AMENDMENTS**

1. (Currently amended) A method for ~~limiting~~ ~~reducing~~ cell aggregation during cell culture to a level wherein no more than 5% of the animal cells in the culture comprise aggregates of at least 5 cells, comprising:

~~during carrying out~~ continuous perfusion culturing of a cell culture comprising cell culture medium and animal cells that easily or inherently form aggregates during culturing, wherein the continuous perfusion culturing comprises:

(a) adding cell culture medium ~~is added~~ to the cell, and

(b) circulating the cell culture ~~is circulated over~~ through a filter module comprising hollow fibers, in an alternating tangential flow resulting in an outflow of cell culture liquid through the pores of the filter module having a lower animal cell density per ml than the cell culture prior to circulating through the filter module,

wherein no more than 5% of the animal cells in the culture form aggregates of at least 5 cells during the continuous perfusion culturing, and wherein the continuous perfusion culturing is continued until animal cells are present in the cell culture at a density of at least  $80 \times 10^6$  viable animal cells/ml.

2-3. (Cancelled)

4. (Currently amended) The method of claim 1, wherein biomass is removed at least once from the cell culture and additional cell culture medium is added to the cell culture to compensate for the biomass removal.

5. (Currently amended) The method of claim 4, wherein the biomass removal is started just before or just after the animal cell density has ~~cells~~ have reached a steady state.

6. (Currently amended) The method of claim 4, wherein a volume of biomass is removed ~~[[of]]~~ is between 2% and 40% of the total volume of the cell culture per day.

7. (Currently amended) The method of claim 1, wherein ~~the alternating tangential flow is achieved using one~~ a first pump ~~[[to]]~~ circulates the cell culture within the filter module comprising hollow fibers and ~~using another~~ a second pump ~~[[to]]~~ removes the liquid having a lower cell density than the cell culture prior to circulating through the filter module ~~the filter separation.~~

8. (Previously presented) The method of claim 1, wherein the animal cells are cultured to a cell viability of at least 90%.

9. (Previously presented) The method of claim 1, wherein no more than 4% of the



animal cells in the culture form aggregates of at least 5 cells during the continuous perfusion culturing.

10. (Canceled)

11. (Previously presented) The method of claim 10, wherein the cells are mammalian cells.

12. (Previously presented) The method of claim 11, wherein the mammalian cells are human cells.

13. (Previously presented) The method of claim 1, wherein the cells produce a biological substance.

14. (Previously presented) The method of claim 13, wherein the biological substance is a protein or a polynucleotide.

15. (Currently amended) The method of claim 13, wherein the biological substance is further purified from the cell culture in downstream processing.